Forest Health Note **February 2006**

2005 *Phytophthora ramorum* Detection Survey and Monitoring in Washington State

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Summary

Phytophthora ramorum, the causal agent of sudden oak death (SOD), ramorum leaf blight, and ramorum dieback, is responsible for widespread tree mortality in central and northern California. Western Washington is at high risk for ramorum leaf blight/shoot dieback due to the presence of known *P. ramorum* hosts in the natural environment, suitable climatic conditions (extended periods of moist weather and mild temperatures), and the presence of nurseries receiving positively identified *P. ramorum* host stock. While Washington's native oak species (Oregon White Oak) is not threatened by *P. ramorum*, Pacific madrone, huckleberry, rhododendron, and Douglas-fir, among many others, are susceptible hosts. Between March 17 and October 25, 2005, one nursery perimeter, twenty-nine general forest, and ten aquatic sites were surveyed and monitored. All foliage samples were screened for *P. ramorum* using DAS ELISA (Double Antibody Sandwich Enzyme-linked immunosorbent assay) and PCR (Polymerase Chain Reaction) based diagnostic tests. All of the 309 foliage samples tested negative for *P. ramorum*.

Introduction

Phytophthora ramorum, the cause of SOD, is responsible for widespread tree mortality in central and northern California. Since its discovery in 1995 on tanoak in Marin County, California, *P. ramorum* has been found in the wildlands of 14 coastal counties in California, Curry County in southern Oregon, and in isolated locations in the United Kingdom and the Netherlands. In 2001, *P. ramorum* was found in a nursery in California, and in 2005 it was detected in nurseries in California, Georgia, Louisiana, Oregon, Tennessee, South Carolina, and Washington.

In 2003, the USDA Forest Service completed a risk assessment pertaining to *P. ramorum*. Based on the number of susceptible hosts, climatic conditions, and locations of nurseries receiving *P. ramorum* host stock, western Washington is considered to be at high risk for ramorum leaf blight/shoot dieback. In addition, shipments of host stock from infected nurseries increase the risk of infection in the perimeters

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of nurseries that receive that stock. The objective of these surveys was to gather information on the distribution of *P. ramorum* in the high-risk areas of Washington.

"Sudden oak death" received its name because of the devastation it has caused on tanoaks, coast live oaks, and other members of the black oak family (*Erythrobalanus*) in California. At this point in time, it is not known to affect members of the white oak family (*Lepidobalanus*), including Oregon white oak, which is the only oak native to Washington. The current list of susceptible (regulated) *P. ramorum* host species that are native to western Washington includes rhododendron, Douglas-fir, bigleaf maple, Pacific madrone, evergreen huckleberry, wood rose, maidenhair fern, pink honeysuckle, false Solomon's seal, and European yew. Other "associated hosts" (found naturally infected, but Koch's postulates have not been completed) native to Washington include grand fir, salmonberry, Pacific yew, poison oak, California hazelnut, California wood fern, Oregon ash, European cranberry bush viburnum, and American cranberry viburnum. Although these species are the only western Washington hosts currently recognized, new host species are being identified as susceptibility trials continue.

Biology

Phytophthora ramorum is a fungus-like organism that can infect a wide range of species in 56 genera. The organism produces microscopic reproductive, dispersal and survival structures making definitive field identification of the disease impossible. Sporangia are the reproductive structures of the organism and are known to play a significant role in spreading the disease. Sporangia may germinate and directly infect plants, but more importantly, they can produce and release zoospores. Zoospores are motile spores (spores with two flagella) that swim in free water and infect plants through wounds or succulent plant tissue. Sporangia are often found on the foliage of hosts and are generally spread by rain splash, or aerially, to new hosts. Chlamydospores are survival structures that can endure months of adverse conditions and germinate when suitable conditions are present. All three structures require free water for germination. Chlamydospores are often present in the soil surrounding infected plants and can be transported with soil on shoes, tires, animal paws/hooves, etc. aiding in long-range dispersal of the organism. Spores can also be transported in stream water.

Symptoms

Symptoms caused by *P. ramorum* vary among host species. *Phytophthora ramorum* primarily infects the leaves of *Rhododendron* spp., although it can also infect branches and may kill entire plants. Infection causes brown-black (necrotic) lesions on portions of the leaf often where water accumulates. The lesions have a diffuse margin and can mimic drought injury. *Phytophthora ramorum* can cause similar symptoms on Pacific madrone and may also cause leaf spots, necrosis along the midvein of the leaf, leaf death, and shoot dieback. Bigleaf maple is a foliar host of *P. ramorum* and symptoms tend to look like scorch starting at the edge of the leaf, but with irregular margins that do not follow the contour of the leaf. Occasional leaf spots have been identified on evergreen huckleberry, but shoot dieback is a more common symptom. *Phytophthora ramorum* causes small lesions on twigs or stems of evergreen huckleberry resulting in death of the infected branch or stem beyond the lesion. *Phytophthora ramorum* also causes cankers on small branches of Douglas-fir resulting in tip dieback, which looks very similar to frost damage. It is important to note that abiotic stressors such as drought and frost, as well as other biological agents, can produce symptoms similar to those caused by *P. ramorum* in the above hosts. Only laboratory analysis of symptomatic tissue can confirm the presence of *P. ramorum*.

Methods

The nursery perimeter and general forest sampling protocol utilized in this survey was developed by the Forest Health Monitoring group within the USDA Forest Service. In forested areas around nurseries that contained suitable host types, four 100-m long transects were distributed along the nursery perimeter so as to sample all available aspects. The transects encompassed the edge of the forested areas along the nursery perimeter and extended 10 meters into the forested area.

In high-risk forest environments, not adjacent to nurseries, a plot center was located at least 100 meters from a road. Four 100-m transects, each 10-m wide, were installed on cardinal azimuths from the plot center. In some cases, general forest surveys were done in state parks, campgrounds, and other high-use areas that are frequented by out-of-state traffic. In these situations, transects were located along the edges of high-traffic areas where suitable hosts were present. A Global Positioning Unit (GPS) was used to identify starting and ending coordinates for each transect.

Along each transect rhododendron, evergreen huckleberry, bigleaf maple, Douglas-fir, Pacific madrone, wood rose, and other known and potential hosts were examined for symptoms of *P. ramorum*. Samples of species not currently recognized as hosts were collected if they exhibited symptoms similar to those exhibited by known hosts because of the likelihood that there are unidentified hosts that will be listed as susceptible in the future. Symptomatic plant tissues were placed in labeled plastic bags and delivered to the laboratory, usually within the same day.

Aquatic monitoring sites were established in March and April of 2005. The aquatic monitoring sites were established in lower watershed perennial streams across western Washington. Two *Rhododendron* leaf traps, each with compartments for five *Rhododendron* leaves, were placed upstream from a bridge, if one was present (Figures 1 and 2). The leaf traps were either secured by a 0.45 kg weight alone or further secured by wrapping a portion of the nylon rope attached to the traps around a tree branch or log. The *Rhododendron* leaf traps were then sampled every two weeks. Freshly collected, non-symptomatic *Rhododendron* leaves were placed into each compartment in the leaf traps and the old *Rhododendron* leaves from each individual trap were pooled into one sample and placed in labeled plastic bags and delivered to the laboratory. Water temperature was measured in º C each time the site was visited.

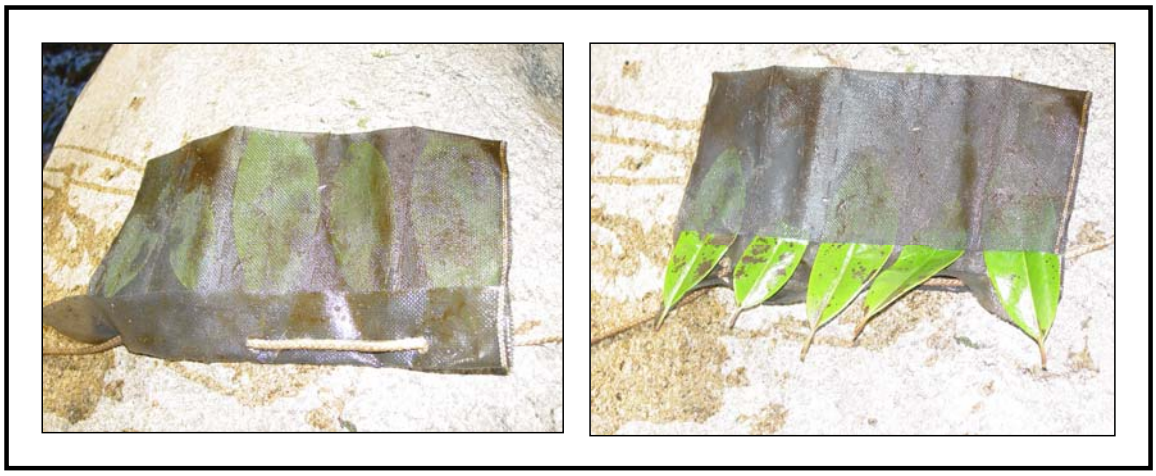

Figure 1. *Rhododendron* leaf traps used in aquatic monitoring sites.

Figure 2. *Rhododendron* leaf traps in perennial stream

The samples were tested by the Washington State Department of Agriculture and the Oregon State University Plant Pathology Clinic. All plant samples were screened for *P. ramorum* using DAS ELISA (Double Antibody Sandwich Enzyme-linked immunosorbent assay) and PCR (Polymerase Chain Reaction) based diagnostic tests.

Results and Discussion

One nursery perimeter and 29 general forested areas were surveyed (Fig. 3). *Phytophthora ramorum* was not isolated from any of the 41 samples collected (Table 1). *Rhododendron* spp. were the most common species sampled, followed by salmonberry (Table 1).

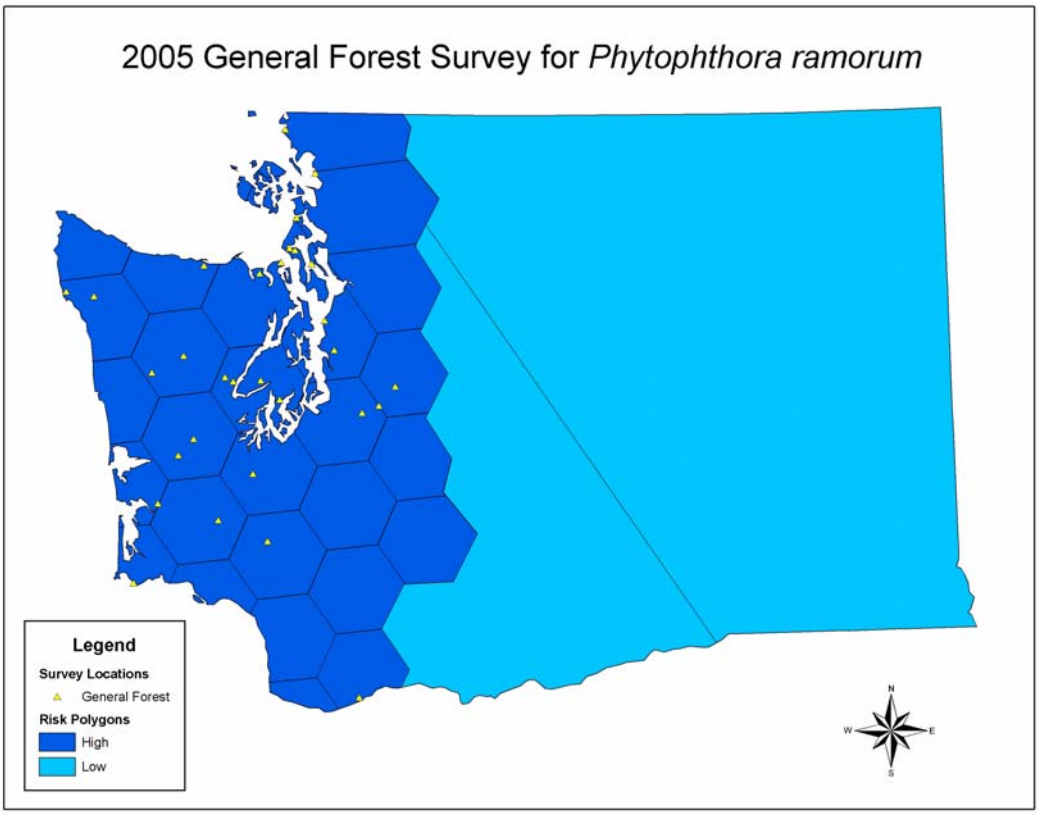

Figure 3. Location of 2005 nursery perimeter and general forest survey sites within USDA Forest Service *P. ramorum* risk polygons. The one nursery perimeter surveyed has been combined with the general forest survey points in this map.

Table 1. Plant species, vegetative material collected, Washington county collection location, and number of samples submitted for analysis for general forest surveys.

Host Species Collected	**Common Name**	**Plant Part**	**Collection Location (County)**	**Number of Samples Submitted**
Acer circinatum Pursh	vine maple	leaf	King, Lewis, Skamania, Whatcom	4
Gaultheria shallon Pursh	salal	leaf	Mason	1
Pseudotsuga menziesii (Mirbel) Franco	Douglas-fir	twig	Pacific, Skamania	2
Rhododendron albiflorum Hook.	Cascade azalea	leaf, twig	Clallam, Grays Harbor, Island, Jefferson, King, Lewis, Mason, Pacific, Pierce, Skamania, Snohomish, Whatcom	16
Rhododendron macrophyllum D. Don ex G. Don	Pacific rhododendron		(combined with data from *R. albiflorum*)	
Rosa woodsii Lindl.	Woods' rose	leaf	Lewis, Mason, Skamania	4
Rubus spectabilis Pursh	salmonberry	leaf, twig	Clallam, Grays Harbor, Jefferson, King, Pacific	9
Umbellularia californica (Hook. & Arn.) Nutt.	California laurel	leaf	Thurston	1
Vaccinium ovatum Pursh	California huckleberry	leaf	Island, Mason	2
Vaccinium parvifolium Sm.	red huckleberry	leaf	Mason, Pacific	2

Ten aquatic sites were monitored throughout the spring, summer, and fall of 2005 (Fig. 4). *Phytophthora ramorum* was not isolated from any of the 268 samples collected.

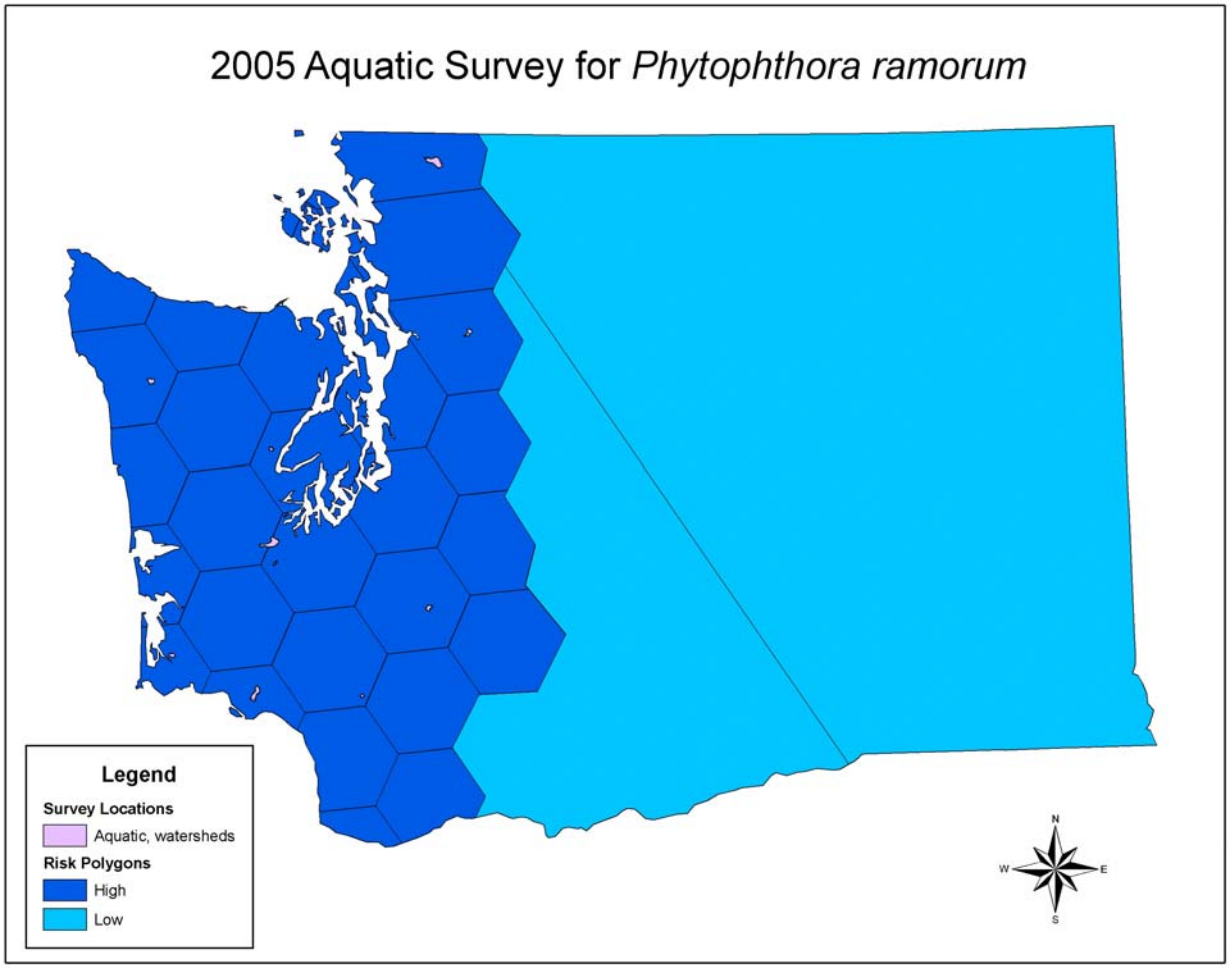

Figure 4. Location of 2005 aquatic monitoring sites within USDA Forest Service *P. ramorum* risk polygons.

While *P. ramorum* has been found in 32 nurseries in the Puget Sound lowlands, there is no evidence that the disease has spread beyond the confines of those nurseries. Infected plants within the nurseries were destroyed to prevent further spread of the pathogen. The organism was brought to Washington nurseries on plants received from other infected nurseries. There is no evidence that the organism has spread naturally in the environment from California or Oregon, to Washington.